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Suitability of Protein-Rich Extract from Okra Seed for Formulation of Ready to Use Therapeutic Foods (RUTF)

* Uhiara, N.S.[1] and Onwuka, Greg[2]

ABSTRACT

Protein-rich extract (PRE) was made from okra (*Abelmuscus esculentus*) seeds. Three versions of feed A, B, and C were formulated using: as sources of protein, A – protein-rich extract from okra seed, B – dehulled and de-fatted okra seed flour (34% protein), C – casein. Each of the feeds was fed to a group of six rats (21 days old mice) for 21 days. The weights of the rats and their droppings were taken at 3-day intervals so were the weights of feed consumed. After 21 days, the rats were decapitated and blood samples harvested. The blood was used for the haematological tests. Liver and heart toxicity indicator including aspartate amino transferase (AST) and alanine amino transferase (ALT) were assayed. Ready-to-use therapeutic food (RUTF) prepared with PRE of okra seeds was formulated and organoleptically assessed in comparison to a standard product. The results showed protein efficiency ratio for A, B, C to be 0.33, 0.14 and 0.47 respectively. The mean weights of the droppings are 2.6, 1.9 and 1.5 respectively, showing significant difference ($p \leq 0.01$). The serum AST was 56.5, 72 and 24 (IU/L) respectively. For ALT it was 16.5, 3.1 and 11 respectively. There was no significant difference between the RUTF as well as weaning foods formulated and the standards in terms of overall acceptability ($p \leq 0.01$).

Keywords: Protein efficiency ratio, toxicity, weaning food, aspartate amino transferase (AST), alanine amino transferase (ALT).

Introduction

Ready-to-use therapeutic food (RUTF) is a homogenous mixture of lipid rich and water soluble foods. It has been shown to be effective in the rehabilitation of severely malnourished children (Manary, 2005). Just like weaning foods, the protein is required not only to be available in good quantity, but must also be of high quality.

RUTF original formulas like F-75 and F-100 which are commercial products, have their protein sourced 100% from milk. Secondly, they come in powdered form and require the addition of water before use. For these reasons, they are mainly used in the treatment of cases of malnutrition for in-patients. In most of the developing countries, most of the hospital patients are out-patients and very high numbers of cases are rarely reported. These necessitated the introduction of new forms of RUTFs. The protein components in this food are sourced from plants. These are easier to handle in terms of contamination and infection (WHO, 1999). Okra seed contains about 25% protein. The amino acid profile of okra seed was found to be similar to that of soyabean. The protein efficiency ratio (PER) was higher for okra seed (Martins *et al.*, 2006). Objectives of this study bothers mainly on cost reduction and the need to create more usage for okra seed, a possible alternative to milk.

[1] Department of Food Science and Technology (FST), Federal Polytechnic Bauchi
[2] Department of Food Science and Technology (FST), Michael Okpara University of Agriculture Umudike (onwukagi@yahoo.com)
* corresponding author: uhiarazi@yahoo.com

Materials and Methods

Three versions of feed A, B and C were formulated having their sources of protein as: A – the protein rich extract from okra seed (Figure 1); B – dehulled and de-fatted okra seed flour (34% protein) and C – casein. Each of the feed was fed to a group of six rats (mice) for 21 days. The three weeks old rats were weighing between 6 – 7 g at the commencement of the experiments. The rats were given 8 g of feed each daily. The rats and droppings weights were determined at 3 days intervals.

At the end of 21 days, the blood of the rats were collected and analyzed under the following parameters: packed cell volume, haemoglobin and white blood cells. Liver toxicity tests were also conducted (Aspartate and Alanin transaminases – AST and ALT inclusive).

Table 1: Formulation of diets fed to rats (15% Protein)

Ingredients (g)	A	B	C (10% Protein)
Protein rich extract	11.4	-	-
Okra seed flour	-	2	-
Casein	-	-	10
Glucose	36	36	12
Multivitamins	8.1	8.1	8.1
Olive Oil	12	12	4
Maize Flour	100	100	70

Formulation of ready-to-use therapeutic food (RUTF)

Using the protein rich extract (PRE) from okra seed (50% protein), groundnut paste, as well as vitamin and mineral mix, the RUTF was produced to give approximately the nutrient composition equivalent of the milk based RUTF – F_{100}.

Table 2: Proximate composition of the protein rich extract

Component	Quantity (%)
Protein	50
Moisture	7
Fat	3
Ash	3
Carbohydrate	37

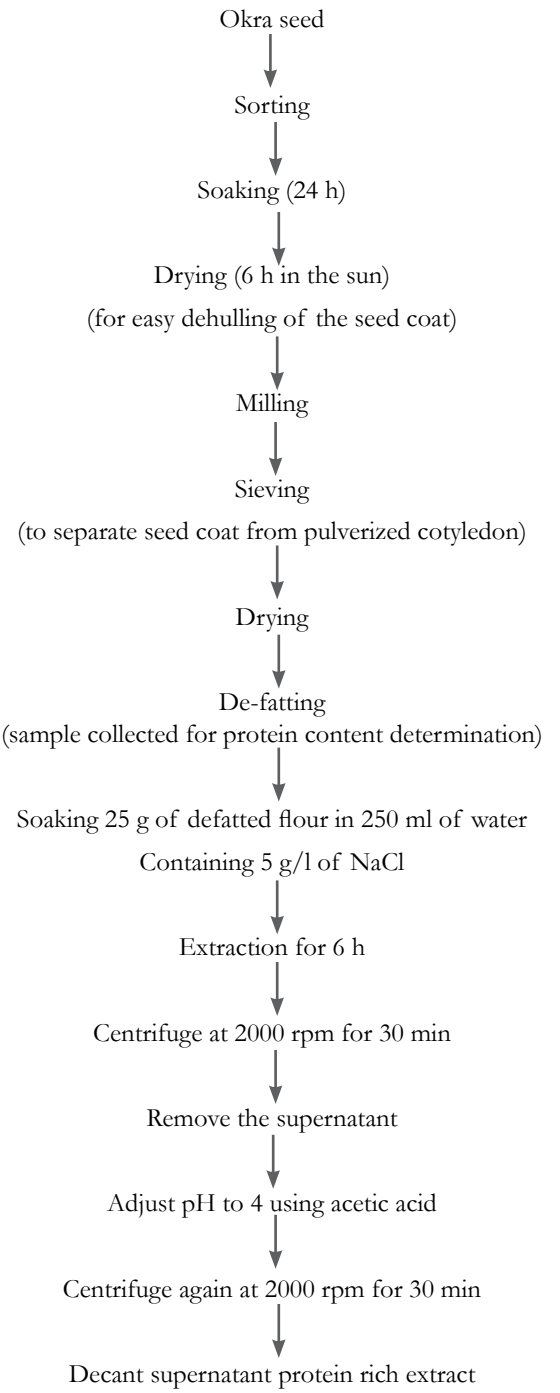

Fig. 1: Production of protein rich extract, PRE (Gilles, 2002)

The yield of protein-rich extract was 7%.

The recipe for the developed RUTF is as follows/100 g

Groundnut paste	-	50g
Protein rich extract from okra seed	-	5g
Malted cereal (Rice)	-	40g
Vitamin Mix	-	2.5g
Mineral Mix	-	2.5g

Energy equivalent

(i) 50 g of groundnut paste = 274 KCal

(ii) 40 g malted cereal [40 x 0.7 (carbohydrate) x 4 KCal/g] = 112

Total [274 + 112] KCal = 386 KCal
(Source: WHO, 1999).

Table 3: Weights of rats (mice) and protein efficiency ratio

Period (3-day interval)	Average Group Weight-Gain of Rats (g) A	B	C
1	2.7 ± 0.4	0.8 ± 0.1	2.2 ± 0.3
2	2.2 ± 0.2	0.6 ± 0.1	1.7 ± 0.4
3	1.8 ± 0.2	0.5 ± 0.2	1.5 ± 0.3
4	1.2 ± 0.4	0.5 ± 0.2	1.0 ± 0.2
5	1.2 ± 0.3	0.4 ± 0.1	1.5 ± 0.3
6	1.1 ± 0.2	0.4 ± 0.1	0.8 ± 0.2
Total Weight gain	9.8	3.2	8.6
Weight Gain/ Period	1.63_a	0.53	1.43_a
Initial Weight	7.1	6.3	6.2
Quantity of Feed Consumed (g)	196	150	180 (10% protein)
Quantity of Protein (g)	29.4	22.5	18
Protein Efficiency Ratio (PER)	0.33	0.14	0.47

Mean ± standard deviation of 6-readings
Figures with same subscript are not significantly different ($p \leq 0.01$)

Sensory evaluation of RUTF and weaning food

The ready-to-use therapeutic food formulated with protein rich extract from okra seed, was compared with Nutty-B from TabatchNick a UN approved formula as standard (Manary, 2005).

A 15-man semi-trained panelist was employed, using a 7-point hedonic scale from most to least preferred. In the same way, weaning food that was maize based with the protein rich extract of okra seed as major source of protein was compared with a commercial formulation – nutrients in terms of colour, mouth feel and general acceptability.

Results and Discussion

The yield in protein rich extracts as well as the protein content of the extracts, vary according to the solubility of such proteins under different conditions of pH, salt concentrations, temperature, pressure, etc. The yield here is 7%, whereas the protein content is 50%. Other workers e.g. Gilles (2002) had figures that varied from 6 – 50% yield as well as protein content. The mean weight gain per period (rate of weight gain), of the rat from Table 3 is 1.63, 0.53 and 1.43 respectively for A, B and C.

There is significant difference in the rate of weight gain of the rats $p \leq 0.01$. The rat in group A fed with okra based protein, apart from gaining weight faster, were physically more active and eat more food from the results in Table 4, the group A rats

Table 4: Weights of rat droppings (g)

Period (3-day Interval)	Groups (Average Group Value) A	B	C
1	2.0 ± 0.2	1.0 ± 0.1	0.8 ± 0.1
2	2.0 ± 0.1	1.5 ± 0.1	1.3 ± 0.3
3	2.5 ± 0.3	2.0 ± 0.4	1.5 ± 0.2
4	3.0 ± 0.6	2.5 ± 0.4	2.0 ± 0.3
5	3.5 ± 0.6	2.5 ± 0.3	2.0 ± 0.2
Mean	2.6^b	1.9^c	1.5^b

* Figures with same subscript are significantly different ($p \leq 0.01$)

also produced significantly more droppings ($p \leq 0.01$). The group C rats however reported a higher protein efficiency ratio, perhaps due to the fact that they consumed less protein and had weight gain second to those of group A. The protein efficiency ratio 0.33 is still a very good one according to Boutrif, (2009), who declared any PER value above 0.27 as excellent. The difference between the A and B feed going by the 0.53 average weight gain and 1.4 protein efficiency ratio of B is wide and quite revealing. The process of extraction must have resulting into more unfolding-denaturation of the parent structures, creating room for improved digestion, absorption and metabolism (Fenemma, 2004).

Table 5: Haematology (blood analysis) of rats

Parameter	Rat Groups (Average Value)			Standards
	A	B	C	
Packed Cell Vol (%) PCV	27 ± 2.0	33 ± 2.0	28 ± 2.0	39 – 49
White Blood Cells (X10g/L)	1.9 ± 1.4	2.4 ± 1.0	2.3 ± 0.3	6 – 15
Lymphocyte (%)	39 ± 3.0	38 ± 3.0	34 ± 3.0	55 – 95
Neutrophil (%)	56 ± 3.0	56 ± 2.0	54 ± 3.0	10 – 40
Monocyte (%)	43 ± 2.0	4.3 ± 1.0	02 ± 0.1	1 – 4*
Eosinophil (%)	01 ± 0.1	1.5 ± 0.3	0	0 – 4
Basophil (%)	0	0	0	0 – 1

Mean ± standard deviation of triplicate reading

Table 6: Toxicity test on experimental rats

Parameter	Rat Groups			Standards
	A	B	C	
Aspartate amino Transferase (AST) (IU/L)	56.5 ± 2.0	72 ± 1.0	24 ± 1.0	45.7 – 80.8
Alanin Amino Transferase (ALT) (U/L)	16.5 ± 0.9	31 ± 1.0	11 ± 0.9	17.5 – 30.2
Albumin (g/L)	41 ± 1.0	41 ± 1.0	38 ± 2.0	38 – 48
Total Protein (g/L)	50 ± 2.0	92 ± 1.0	46 ± 2.0	56 – 76
Conjugated Bilirubin (mmol/L)	18 ± 0.9	12 ± 0.9	3 ± 0.2	(2-5)0.2 – 0.55mg/dL
Total Bilirubin (mmel/L)	28.5 ± 2.0	40 ± 2.0	18 ± 2.0	(25-30)15 – 21mg/dL
Alkaline phosphate ALP (IU/L)	39 ± 0.9	39 ± 0.9	33 ± 1 .0	56.8 – 128

Mean ± standard deviation of triplicate reading

In almost all the haematological parameters examined there was no real toxicity treats discernable for the okra seed protein or the whole okra seed. In PCV, haemoglobin counts (Table 5) the figures are safely within the standards (IOWA, 2013).

ALT and AST are members of the transaminase family enzymes. They are found mainly in the liver and heart respectively. When these organs are damaged, additional ALT and AST are released into the blood stream. Their amounts in the blood are directly related to the extent of the tissue damage (Dufour *et al.,* 2000).

Table 7: Results of sensory evaluation

Sample	Colour	Mouth feel	Overall Acceptability
A	6a	6a	7b
CA	5a	7b	6b
B	7c	7d	7a
CB	6c	6d	7c

Figures with same letter are significantly different $p \leq 0.05$

A: Okra seed protein based RUTF
CA: RUTF control
B: Okra seed protein based weaning food
CB: Control weaning food

Considering the AST (56.5IU/L) and ALT (16.5 IU/L) values, the okra protein feed compared to the standard range (45.7 – 80.8) AST and (17.5 – 30.2) ALT, look quite safe. Even those of whole okra seed are not condemned as toxic. The result of the sensory evaluation of the RUTF as well as weaning foods based on the okra seed protein shown on Table 7, indicates the products were well accepted. ($p \leq 0.01$).

Conclusion and Recommendations

The efficiency ratio of 0.33 puts the quality of okra seed protein on the high side (Boutrif, 2000). The key toxicity indications – serum AST and ALT from Table 6 does not portray okra seed protein extract as toxic. Enriching foods with okra seed protein is a cheap and safe way of putting to use okra which is one of the few crops that grows in every part of Nigeria.

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